

ANTI-MICROBIAL-ADHESION FRACTIONDERIVED FROM VACCINIUM

CROSSREFERENCE TO RELATED APPLICATIONS

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This application is a Continuation-In-Part of United States Serial Number 08/772,021, filed December 19, 1996.

BACKGROUND OF THE INVENTION

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TECHNICAL FIELD

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This invention relates to plant extracts having therapeutic and other uses, and more specifically to an extract of juice from berries of the *Vaccinium* plant genus having an anti-microbial-adhesion activity.

BACKGROUND ART

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Adhesion of the bacteria to each other (intraspecies) and to other bacterial species (intergeneric coaggregation) as well as to host tissues and cells contributes significantly to disease progression and pathology as for example in dental caries and plaque as well as in the persistance of *Helicobacter pylori* infection. It would therefore be useful to have

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compounds which can interrupt microbial adhesion and aggregation.

Chronic ulcers have been shown to be caused by *Helicobacter pylori* and the presence of chronic ulcers is associated frequently with complications leading to gastric cancer [Cover and Blaser, 1995]. *H. pylori*, first isolated from a specimen of gastritis in 1983, is a gram-negative, spiral microaerophilic, fastidious organism. Generally all patients with gastric ulcer have *H. pylori* and eradication of the bacteria is followed by the resolution of gastritis [Cover and Blaser, 1995; Blaser, 1996]. It is estimated that *H. pylori* infection increases the risk of gastric cancer by approximately six times. Epidemiological studies showed that about 50% of the world population is infected with *H. pylori* (80% and 40% of adults in developing and developed countries, respectively) but only part of the infected population develop clinical symptoms.

The unique characteristic of *H. pylori* infections is persistence of the agent within the gastric mucous. The immune response against the infection is not able to eradicate the infection [Dunn et al, 1997]. Histological examination shows that the bacteria are in close association with mucus layer and underneath gastric cells [Lee et al, 1993]. The microorganisms produce a number of adhesins which mediate attachment of the bacteria to

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cognate receptors present in gastric mucus and epithelium
[Boren and Falk, 1995; Dunn et al, 1997]. They
agglutinate erythrocytes and it has been shown that the
ability to adhere to gastric epithelium correlates with
5 its ability to agglutinate red blood cells. Adhesion to
gastric epithelial cells and to the mucus layer coating
such surfaces is considered to be the most important
factor enabling the pathogen to cause persistent
infection [Boren and Falk, 1995]. It is an object of the
10 present invention therefore to provide anti-adhesion
compounds for anti-adhesion therapy which can be an
alternative to the conventional antibiotic treatment.

Bacterial activity of over 500 different bacteria
have been implicated in both human dental plaque and
15 caries (cavities). Adhesion of the bacteria to each
other (intraspecies) and to other bacterial species
(intergeneric coaggregation) as well as to oral surfaces
is one of the major factors leading to dental plaque as
well as carries and periodontal diseases.

20 It would be useful to have additional anti-
aggregation medicaments for use in oral hygiene. United
States patent 5,362,480, columns 1-3 provides a
discussion on bacterial adhesions and oral hygiene. Ofek
and Doyle, 1994 provides a general discussion of
25 bacterial adhesion incorporated herein by reference in
their entirety.

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Briefly, microbial accumulations on the tooth surfaces, termed dental plaque, are the causative agents of both dental caries and periodontal diseases [Slots, 1977; Socransky et al., 1982; Savit and Socransky, 1984; 5 Dzink et al., 1985, 1988]. The adhesion of bacteria to the pellicle-coated tooth surface appears to be the first step in the formation of dental plaque [Gibbons and van Houte, 1975; van Houte, 1980]. Oral streptococci and to some extent *Actinomyces* sp. are the prominent early 10 colonizers of the tooth surfaces [Nyvad and Kilian, 1990] and apparently attach to macromolecules selectively adsorbed to tooth surfaces [Gibbons et al., 1991].

Microorganisms that progressively accumulate thereafter, mostly gram negative anaerobic bacteria, in 15 the gingival crevice area, are the late colonizers and are believed to play a central role in the initiation and progression of periodontal diseases. In this second step the bacteria co-aggregate or adhere to each other. The primary constituents of dental plaque are bacteria in a 20 matrix composed of extracellular bacterial polymers and salivary products. The bacterial species present in dental plaque are heterogeneous and they change progressively as the clinical condition goes from normal health through gingivitis to advanced stages of 25 periodontitis [Moore and Moore 1994].

Studies *in vitro* of coaggregation among oral
bacteria revealed that coaggregation is essentially the
result of adhesion mediated by specific interactions
between complementary molecules on the surfaces of the
5 participating bacteria [Kolenbrander et al, 1993].

Several hundreds of oral bacterial pairs were found to
participate in this type of multigeneric coaggregation
reactions *in vitro*, but only for handful of pairs the
molecular mechanisms have been characterized [Ofek and
10 Doyle, 1994]. In many cases the coaggregation involves
lectin-carbohydrate interaction whereby the sugar
residues on one bacterial pair interact with a lectin on
the surface of the other bacterial pair.

Based on the ability of simple and complex sugars to
15 inhibit coaggregation, a number of distinct specificities
are now recognized including lactose, sialic acid,
rhamnose and fucose inhibitable coaggregations. It
should be noted however, that still a large number of
coaggregating pairs are not inhibited by any of the
20 carbohydrates tested and therefore they may have a
distinct specificity involving surface constituents other
than lectin and carbohydrate [Ofek and Doyle, 1994].

It is therefore an object of the present invention
to provide compounds to inhibit interbacterial
25 coaggregation or adhesion of oral bacteria or to reverse
existing coaggregation.

There is presently anecdotal and scientific evidence that cranberry juice or some fraction thereof inhibits or reduces bacterial infections of the bladder [Avorn et al, 1994], restricted to P-fimbriated bacteria. Currently,
5 it is believed that this action is due to interruption of the adhesion of P-fimbriated bacteria to mammalian cells.

United States patents 5,002,759 and 5,362,480 disclose anti-adhesion compositions that can be used in treating oral bacteria. However, neither of these
10 patents disclose compositions from *Vaccinium*, and in particular cranberry or blueberry, and are not the composition of the present invention.

United States patent 5,185,153 provides a composition for use in oral compositions for the lysis
15 and killing of oral bacteria. The '153 patent does not derive the agent from cranberry and is not the composition of the present invention.

United States patent 5,474,774 to Walker et al issued December 12, 1995 does disclose an extract from
20 cranberry which is enriched for an activity which inhibits bacterial adhesion to surfaces. However, the extract is not the composition of the present invention as shown in comparative Example 5 herein below. Further, the method of the '774 patent initiates the extraction
25 from whole cranberries with multiple extraction steps.
PCT/US96/03978 (WO 96/30033) published application

further discloses an extract/composition. However, as shown herein below the composition of PCT/US96/03978 is not the composition of the present invention as shown in comparative Example 5 herein below.

5 United States Patent 4,857,327 to Virdalm discloses a preparation isolated from the remainder of the berries after the pulp flesh has been removed. In the art of juice making the material from the Virdalm '327 is referred to as the "press cake" which is not used for making juice. Juice is derived from the "pulp flesh".
10 Therefore since the starting point for the Virdalm '327 product is from the remainder after discarding the pulp flesh and thereby the juice.

United States Patent 5,683,678 to Heckert et al
15 discloses an oral composition containing anthocyanins isolated from cranberries. However, the extract is not the composition of the present invention as shown in comparative Example 5 herein below.

It is an object of the present invention to use
20 commercially prepared juice or concentrate from berries of the *Vaccinium* plant genus as the starting source of isolation with minimal extraction steps of an anti-coaggregation, adhesion fraction.

SUMMARY OF THE INVENTION

According to the present invention, a non-food composition comprising a suitable carrier and an effective amount of an aggregation/adhesion inhibitory fraction isolated from juice from berries of the *Vaccinium* plant genus is provided. In an embodiment the anti-aggregation fraction is isolated from cranberry juice. Further, the invention includes a method of inhibiting interbacterial adhesion including mucosal adhesion of *Helicobacter pylori*, coaggregation of oral bacteria, and reversing adhesion of oral bacteria by treating with the isolated fraction from cranberry juice and a pharmaceutically acceptable carrier.

The anti-aggregation/adhesion water extract fraction is characterized as being polymeric and having a molecular weight $\geq 14,000$; an elemental analysis of carbon 43-51%, hydrogen 4-5%, no nitrogen, no sulfur and no chlorine; an NMR line spectrum as set forth in Figures 2A and 2B; and an ultraviolet spectrum with an absorption peak at 280 nm in neutral or acidic pH solution which is absent in alkali solutions. This fraction exhibits adhesion inhibitory activity against P fimbriated bacteria, oral bacteria as well as reversal of aggregation/adhesion of oral bacteria and adhesion inhibitory activity against *Helicobacter pylori*.

The present invention further provides two methods of extracting the composition of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

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Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

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FIGURE 1 is a schematic diagram of the isolation of the adhesion-inhibitory active fraction from cranberry juice wherein the starting amount is 1.5 liters of cranberry juice cocktail or 25 ml of concentrated

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cranberry juice;

FIGURE 2A-2C are NMR line spectra of the adhesion-inhibitory active fraction from cranberry juice, wherein (A) is PF-1 (proton NMR), (B) is PF-1 after removal of bound iron (proton NMR), and (C) is acid-hydrolysis

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product (carbon NMR);

FIGURE 3 is a schematic diagram of an ELISA to determine anti-adhesion activity of cranberry fractions against P-fimbriated bacteria;

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FIGURE 4 is a schematic diagram of method to determine reversal of adhesion (coaggregation) and method

to show inhibition of adhesion (coaggregation) of oral bacteria;

FIGURE 5 is a graph showing the results from an ELISA titrating anti-PF-1 antibody;

5 FIGURE 6 is a graph of prior art results showing correlation between inhibitory activity against type 1 fimbrial adhesion of *E. coli* and fructose content of various juices;

10 FIGURE 7 is a standard curve to estimate number of bacteria per well from ELISA values; and

FIGURE 8 is a graph showing the percent inhibition by PF-1 of adhesion of P-fimbriated *E. coli* to erythrocyte membranes (ghosts).

15 FIGURE 9 is a graph showing the inhibition by NDM of adhesion of *H. pylori* to human gastric musin. Human mucin (0.1ml, 1mg/ml protein in saline) was immobilized on the bottom of microtiter plates and exposed to *Helicobacter* suspension in PBS or PBS containing 0.1mg/ml NDM. After washing off non bound bacteria, the amount of
20 mucin-adherent bacteria was quantitated by monitoring the urease activity of these bacteria over time, as determined by calorimetric assay of urea hydrolysis at OD 660.

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DETAILED DESCRIPTION OF THE INVENTION

5 The present invention provides a non-food composition comprising a suitable carrier and an effective amount of the isolated adhesion inhibitory water extract fraction from juice isolated from the pulp flesh of berries of the plant genus *Vaccinium* as the active ingredient for use in oral hygiene and as a gastro-intestinal/enteric therapeutic. In one embodiment 10 the isolated adhesion inhibitory fraction is designated as PF-1 and is characterized by:

being a polymeric compound having a molecular weight $\geq 14,000$; and

15 exhibiting adhesion inhibitory activity against oral bacteria, *H. pylori*, and including coaggregation inhibition and reversal of coaggregation.

The active fraction is further characterized as:

having an elemental analysis of carbon 43 - 51%, hydrogen 4 - 5%, no nitrogen, no sulfur and no chlorine;

20 having a characterizing NMR line spectrum as set forth in Figures 2A and 2B;

having an ultraviolet spectrum with an absorption peak at 280 nm in neutral or acidic pH solution which is absent in alkali solutions; and

25 exhibiting microbial-adhesion-inhibitory activity.

5 The composition of the present invention can be
administered to a patient in need of such a composition for
treating *H. pylori* infections (see Example 9 and 10
herein) as an active ingredient and a pharmaceutically
acceptable carrier or diluent as well as for oral
hygiene. Generally, the concentration of the isolated
adhesion inhibitory fraction is between 1 μ g and 10 mg
per milliliter.

10 In a preferred embodiment the fraction is prepared
from cranberry juice or juice concentrate. However,
other species of the plant genus *Vaccinium* can be used in
the practice of the present invention such as, but not
limited to, bilberry and blueberries [Ofek et al, 1993,
page 198, Table 2].

15 Oral hygiene refers to the control of dental plaque,
dental caries and periodontal disease (gingivitis and
periodontitis).

20 Anti-*H. pylori* therapeutic refers to the control of
H. pylori gastric infection by anti-adhesion compounds
for anti-adhesion therapy. It further refers to the
inhibition of adhesion of *H. pylori* to human mucin as
shown herein in the Examples.

25 Adhesion refers to the general aggregation of
bacteria to each other, to other cell surfaces and to non
cell surfaces generally through adhesion molecules on the
surface of the bacteria. Further as used herein

coaggregation refers to the aggregation/adhesion of two or more bacteria, including bacteria of different species, and coaggregation reversal refers to reversing the aggregation or adhesion (Figure 4) between the bacteria. Inhibition of coaggregation or adhesion generally refers to prevention of the initial adhesion or aggregation of the bacteria. In general, anti-aggregation is used to refer to either or both inhibition and reversal of coaggregation, both intra- and inter-bacterial species, as indicated by the context of the use.

Coaggregation inhibition as used herein with reference to oral bacteria will have the *in vivo* effect of (i) prevention of accretion of new organisms to the already formed aggregates in the dental plaque and (ii) preventing the re-accumulation and recolonization of bacteria that have been just removed by the tooth brush or other means allowing elimination from the oral cavity by the salivary flow/rinsing. If not prevented they can reattach to the clean tooth surface and/or the remaining dental plaque.

Reversal of coaggregation as used herein with reference to oral bacteria will have the *in vivo* effect of actively disrupting/dislodging or dispersing the existing dental plaque both on the tooth surface and on the mucosal surfaces.

Coaggregation inhibition and reversal of
coaggregation as used herein with reference to *H. pylori*
will have the *in vivo* effect of (i) prevention of
accretion of new organisms to the already formed
5 aggregates and (ii) preventing the re-accumulation and
recolonization of bacteria on the mucosal surfaces and
(iii) interruption of the adhesion of the bacteria with
mucus layer and underneath gastric cells and interruption
of their adhesins which mediate attachment of the
10 bacteria to cognate receptors present in gastric mucus
and epithelium.

P-fimbrial adhesion molecules bind specifically to a
group of receptors identified as P-blood group antigens.
The receptor(s) are present on the surface of various
15 types of human cells - among other - urinary tract
epithelium and red blood cells, and mediates attachment
of the bacteria and subsequent colonization of the
epithelium of the urinary tract. P-fimbriated *E. coli*
cause agglutination (HA) of human red blood cells (RBC)
20 [Ofek and Doyle; 1994].

The isolated adhesion inhibitory fraction PF-1 is
insoluble in butanol and ethylacetate and is acid
precipitable. It can be redissolved in water without
loss of activity. The fraction loses partial activity
25 upon heating in acidic solutions. It has a positive

reaction in a phenol-sulfuric acid test as described in the Examples set forth herein below.

5 The present invention provides a method of inhibiting and reversing intergeneric coaggregation/adhesion of bacteria by treating with PF-1 (or PF-2 or NDM), and a pharmaceutically acceptable carrier. The concentration of PF-1, in the carrier is between 1 $\mu\text{g/ml}$ and 10 mg/ml with a preferred embodiment between 10 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$. For inhibition of
10 coaggregation a range between 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ has been shown to be effective. For reversal of coaggregation a range between 100 $\mu\text{g/ml}$ and 250 $\mu\text{g/ml}$ has been shown to be effective. For NDM the equivalent PF-1 units contained in the preparation are determined and the
15 concentration adjusted accordingly. For PF-2, the PF-1 comparative unit is determined and the concentration adjusted accordingly (See Table 8).

In the method of treating oral bacteria the inventive compositions may constitute an integral part of
20 a toothpaste, dental cream or gel, tooth powder, or mouthwash and applied during the regular brushing, or the compositions may be formulated and packaged as a separate treatment and applied separately before, after, and/or in between regular brushing times. The compositions may be
25 applied by brushing, rinsing, chewing, and with active oral irrigation systems and any other means known in the

art. Further chewing gums and lozenges as are known in the art may be used.

5 In the method of treating *H. pylori* infection in patients with such infections or with other bacterial infections that would benefit from the composition, the anti-adhesion fraction, PF-1, PF-2 or NMD, can be administered in various ways suitable for gastro-intestinal therapy. It should be noted that it can be administered as the compound or as pharmaceutically acceptable salt and can be administered alone or as an active ingredient in combination with pharmaceutically acceptable carriers, diluents, adjuvants and vehicles. The composition will generally be administered orally. Conventional methods such as administering the compounds in tablets, suspensions, solutions, emulsions, capsules, 15 powders, syrups and the like are usable. Known techniques which deliver the anti-adhesion composition orally or intravenously and retain the biological activity are preferred.

20 Formulations that can be administered subcutaneously; topically or parenterally or intrathecal and infusion techniques are also contemplated by the present invention as well as suppositories and implants. The pharmaceutically acceptable carriers, diluents, 25 adjuvants and vehicles as well as implant carriers generally refer to inert, non-toxic solid or liquid

fillers, diluents or encapsulating material not reacting with the active ingredients of the invention. The pharmaceutical formulations suitable for injection include sterile aqueous solutions or dispersions and
5 sterile powders for reconstitution into sterile injectable solutions or dispersions. The carrier can be a solvent or dispersing medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like),
10 suitable mixtures thereof, and vegetable oils.

Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Nonaqueous vehicles such
15 a cottonseed oil, sesame oil, olive oil, soybean oil, corn oil, sunflower oil, or peanut oil and esters, such as isopropyl myristate, may also be used as solvent systems for compound compositions. Additionally, various additives which enhance the stability, sterility, and
20 isotonicity of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens,
25 chlorobutanol, phenol, sorbic acid, and the like. In many cases, it will be desirable to include isotonic

agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. According to the present invention, however, any vehicle, diluent, or additive used would have to be compatible with the compounds.

Examples of delivery systems useful in the present invention include: U.S. Patent Numbers 5,225,182; 5,169,383; 5,167,616; 4,959,217; 4,925,678; 4,487,603; 4,486,194; 4,447,233; 4,447,224; 4,439,196; and 4,475,196. Many other such implants, delivery systems, and modules are well known to those skilled in the art.

In Example 1 set forth herein below, a method of isolating a fraction from cranberry juice, as the exemplar species of *Vaccinium*, exhibiting adhesion inhibitory activity against P fimbriated bacteria and oral bacteria is described. The method includes the steps of dialyzing cranberry juice, or a juice concentrate, extensively against double distilled water using dialysis tubing with a 12,000 - 14,000 molecular weight cut-off. The non-dialyzable material (NDM) remaining in the dialysis tubing is then lyophilized. The lyophilized NDM is then fractionated on a polyacrylamide resin column and the active fraction is eluted from the column with water, lyophilized, and

designated PF-1 as shown in Figure 1. In the Examples herein either the NDM or PF-1 fractions are used as indicated.

Further, in Example 8, additional
5 preparation/purification steps are provided. When eluting the resin column 0.1M ammonia can be used. Applicants have determined that some batches of resin provide better yields when eluted with ammonia than water as is known in the art.

10 NDM as shown in the examples can be used in the present invention to inhibit or reverse intergeneric coaggregation/adhesion of oral bacteria, and a pharmaceutically acceptable carrier. The concentration of NDM however in the carrier is between 25 μ g/ml and 100
15 mg/ml. For inhibition of coaggregation a range between 0.05 mg/ml and 0.4 mg/ml can be used. For reversal of coaggregation a range between 1 mg/ml and 4 mg/ml can be used.

The present invention also provides for an antibody
20 directed against the isolated adhesion inhibitory fraction, PF-1, from cranberry juice. The antibody can be either polyclonal or monoclonal.

The antibodies are prepared against the isolated PF-1 (or portions of PF-1 that may be isolated as well as
25 from NDM and PF-2) used as the immunogen. The material can be used to produce antibodies by standard antibody

production technology well known to those skilled in the art, as described generally in Harlow and Lane,

Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988 and Borrebaeck,

5 *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992.

For producing polyclonal antibodies, a host, such as a rabbit or goat, is immunized with PF-1, generally with an adjuvant and, if necessary, coupled to a carrier;

10 antibodies to PF-1 are collected from the sera. More particularly, antibodies against PF-1 were prepared in rabbits by inoculation with mixtures containing formalin-killed bacteria of *E. coli* P-fimbriated (strain IHE-1002; in some reports strain is designated IHE or IHI) and PF-

15 1. The mixtures are incubated for 1 hour at 37°C to allow bacteria to absorb the substance. Immunization was carried out by intravenous injections of the mixtures containing increasing concentrations of bacteria and PF-1 (10^8 - 10^9 bacteria and 0.25-5 mg/ml of PF-1/injection)

20 three times a week, for four weeks. Two weeks after the last injection, the serum is collected and analyzed by ELISA.

For producing monoclonal antibodies, the technique involves hyperimmunization of an appropriate donor with

25 the PF-1 as above, generally a mouse, and isolation of splenic antibody producing cells. These cells are fused

to a cell having immortality, such as a myeloma cell, to provide a fused cell hybrid which has immortality and secretes the required antibody. The cells are then cultured, in bulk, and the monoclonal antibodies
5 harvested from the culture media for use.

The antibody can be bound to a solid support substrate or conjugated with a detectable moiety or be both bound and conjugated as is well known in the art. (For a general discussion of conjugation of fluorescent
10 or enzymatic moieties see Johnstone & Thorpe, *Immunochemistry in Practice*, Blackwell Scientific Publications, Oxford, 1982.) The binding of antibodies to a solid support substrate is also well known in the art. (see for a general discussion Harlow & Lane
15 *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, New York, 1988 and Borrebaeck, *Antibody Engineering - A Practical Guide*, W.H. Freeman and Co., 1992) The detectable moieties contemplated with the present invention can include, but are not limited
20 to, fluorescent, metallic, enzymatic and radioactive markers such as biotin, gold, ferritin, alkaline phosphatase, β -galactosidase, peroxidase, urease, fluorescein, rhodamine, tritium, ^{14}C and iodination.

ELISAs are immunoassays which can be employed to
25 identify and titer the anti-PF-1 antibody as well as the amount of PF-1 in a preparation. ELISA assays are well

known to those skilled in the art. Both polyclonal and monoclonal antibodies can be assayed with an ELISA.

Where appropriate other immunoassays, such as radioimmunoassays (RIA) and immunoblots, can be used as

5 are known to those in the art. Available immunoassays are extensively described in the patent and scientific literature. See, for example, United States patents 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 10 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521 as well as Sambrook et al, *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor, New York, 1989.

The present invention provides for a composition comprising an effective amount of an isolated adhesion 15 inhibitory fraction from *Vaccinium*, in a preferred embodiment the isolated adhesion inhibitory fraction from cranberry juice, PF-1, and a pharmaceutically acceptable carrier which does not react with the active ingredients of the invention and which does not decrease the 20 biological activity of the present invention.

The composition is administered and dosed in accordance with good medical practice, taking into account the clinical condition of the individual patient, the site and method of administration, scheduling of 25 administration, patient age, sex, body weight and other factors known to medical practitioners. The

pharmaceutically "effective amount" for purposes herein is determined by such considerations as are known in the art. The amount must be effective to achieve improvement including but not limited to inhibition and/or reversal of oral intra- and inter-bacterial species coaggregation as described in the Examples herein below and to improvement or elimination of symptoms and other indicators as are selected as appropriate measures by those skilled in the art.

The present invention provides a non-food composition which comprises a suitable carrier and an isolated adhesion inhibitory fraction from *Vaccinium*. In a preferred embodiment an effective amount of the isolated adhesion inhibitory fraction PF-1 is used. However, in an alternative embodiment NDM can be used.

The preferred oral hygiene compositions of the present invention are in the form of toothpaste (dental cream, gel or tooth powder), as well as mouthwash, pre-brushing, or post-brushing rinse formulations, chewing gums and lozenges.

Ingredients typically included in toothpastes and gels may be used in toothpaste and gel compositions in accordance with the invention as are known in the art. Suitable ingredients include abrasive polishing materials, sudsing agents, flavoring agents, humectants,

binders, sweetening agents, and water and are described generally herein below.

Mouthwashes are typically comprised of a water/alcohol solution, flavor, humectant, sweetener, foaming agent, and colorant.

Abrasives which may be used in the compositions of the invention include alumina and hydrates thereof, such as alpha alumina trihydrate, magnesium trisilicate, magnesium carbonate, aluminosilicates, such as calcined aluminum silicate and aluminum silicate, calcium carbonate, zirconium silicate, polymethyl methacrylate, powered polyethylene, silica xerogels, hydrogels and aerogels and the like. Also, suitable as abrasive agents are calcium pyrophosphate, insoluble sodium metaphosphate, calcium carbonate, dicalcium orthophosphate, particulate hydroxyapatite and the like. Depending on the form which the oral composition is to take, the abrasive may be present in an amount of from 0 to 70% by weight, preferably 1 to 70% by weight, more preferably from 10 to 70% by weight, particularly for toothpastes.

Humectants contemplated for use in the present invention include glycerol, polyol, sorbitol, polyethylene glycols, propylene glycol, hydrogenated partially hydrolyzed polysaccharides and the like. The humectants are generally present in amounts of from 0 to

80%, preferably 5 to 70% by weight, particularly for toothpastes. Thickeners may be present in toothpaste creams and gels at 0.1 to 20% by weight.

Binders suitable for use in the compositions of the invention include hydroxyethyl cellulose (Natrosol®), sodium carboxymethyl cellulose and hydroxypropyl cellulose (Klucel®), as well as xanthan gums, Irish moss and gum tragacanth. Binders may be present in the toothpaste of the invention to the extent of from 0.01 to 10%.

Suitable foaming agents include soap, anionic, cationic, nonionic, amphoteric and/or zwitterionic surfactants. These may be present at levels of 0 to 15%, preferably 0.1 to 15%, more preferably 0.25 to 10% by weight.

Certain pyrophosphate and other polyphosphate salts have been disclosed in U.S. Patent Nos. 4,515,772 and 4,627,977 as being useful as anti-calculus agents. These include di- and tetra-alkali metal pyrophosphates wherein the alkali metals are preferably selected from the group consisting of sodium and potassium. Polyphosphate salts may be included generally in the amount such that it provides for at least 0.5% polyphosphate anions, the upper level being about 10%, preferably about 7.5%.

Various anionic polymers may be employed as anticalculus and/or antiplaque agents. Suitable polymers

include carboxylate polymers, sulfonate polymers,
polymers containing a sulfonate and a carboxylate moiety,
carboxylate polymers containing phosphinate units, and
mixtures thereof. Some carboxylate polymers suitable in
5 the present compositions are described by Gaffar et al.,
U.S. Patent No. 4,808,400, incorporated by reference
herein. Other carboxylate polymers containing mono- or
disubstituted hypophosphite units along the polymer
backbone are described in a U.S. Patent No. 5,011,682
10 incorporated by reference herein. The anionic polymers
may be included at a level from about 0.01 to about 10%,
preferably from about 0.05 to about 5%.

Zinc salts are disclosed as anti-calculus and anti-
plaque agents in U.S. Patent No. 4,100,269 and in U.S.
15 Patent Nos. 4,416,867, 4,425,325 and 4,339,432.
Preferred compositions of the invention include zinc
salts, particularly zinc citrate. The zinc compounds may
be present in the compositions in amounts sufficient to
furnish about 0.01% to about 4% zinc, or preferably about
20 0.05% to about 1%, zinc ion.

Fluoride sources used in toothpastes such as sodium
fluoride, stannous fluoride, sodium monofluorophosphate,
zinc ammonium fluoride, tin ammonium fluoride, calcium
fluoride and cobalt ammonium fluoride may be, and
25 preferably are, included for delivering anti-carries
benefit. Preferred compositions of the invention include

the fluoride source. Fluoride ions are typically provided at a level of from 0 to 1500 ppm, preferably 50 to 1500 ppm, although higher levels up to about 3000 ppm can be used.

5 Sweeteners suitable for use in the present dentifrice, preferably at levels of about 0.1% to 5%, may include saccharin and other non-caloric sweeteners as known in the art. Flavors are usually included in toothpastes in low amounts, such as from 0.01 to about 5%
10 by weight, especially from 0.1% to 5%. Titanium dioxide is a suitable whitener but others known in the art may be used. Dyes/colorants suitable for dentifrices, i.e., FD&C Blue #1, FD&C Yellow #10, FD&C Red #40, etc., can be employed in the dentifrices of the invention or others
15 known in the art.

Water-soluble antibacterial agents, such as chlorhexidine digluconate, hexetidine, alexidine, quaternary ammonium anti-bacterial compounds and water-soluble sources of certain metal ions such as zinc,
20 copper, silver and stannous (e.g., zinc, copper and stannous chloride, and silver nitrate) can also be included.

Various other optional ingredients may be included in the compositions of the invention, such as
25 preservatives, vitamins such as vitamin C and E, other anti-plaque agents such as stannous salts, copper salts,

strontium salts and magnesium salts. Also included may be pH adjusting agents, anti-carries agents such as urea, calcium glycerophosphate, sodium trimetaphosphate, silicone polymers, plant extracts, desensitizing agents for sensitive teeth such as potassium nitrate and potassium citrate, and mixtures thereof.

Casein and/or its hydrolysate may be included as anticaries agents, e.g. at a level of 0.01 to 20% by weight, preferably 0.1 to 10%.

The corresponding compounds mentioned above which are used in toothpastes, are generally suitable within the ranges above for mouthwashes as well. The mouthwash can include ethanol at a level of from 0 to 60%, preferably from 5 to 30% by weight.

The present invention also provides for a fortified food composition for oral hygiene comprising a suitable food carrier and an effective amount of an isolated adhesion inhibitory fraction from *Vaccinium*. In a preferred embodiment the food carrier is a fruit juice and the isolated adhesion inhibitory fraction is PF-1. The food carrier is selected such that it does not decrease the biological activity of the present invention. The concentration of the adhesion inhibitory fraction in the food carrier is between 10 μ g/ml and 10 mg/ml or the equivalent weight/volume concentration for non-liquid foods.

The ability of cranberry products to inhibit *E. coli* adhesion to surfaces such as bladder cells was shown as early as 1984 by Sobota [Sobota, 1984; Schmidt and Sobota, 1988]. Applicants have confirmed these results with Ocean Spray cranberry juice cocktail [Zafriri et al, 1989; Ofek et al, 1991; Ofek et al, 1993]. Applicants confirmed that fructose inhibits adhesion of *E. coli* mediated by type 1 fimbriae (Fig. 6 [Ofek et al., 1993]).

An inhibitor, PF-1, was also found that is a polymeric substance which inhibits mannose resistant adhesion of urinary isolates of *E. coli* to surfaces such as human erythrocytes. The specificity of adhesion of some of these isolates was mediated by P fimbriae. PF-1 has, however, no effect on adhesion of diarrhoeal isolates of *E. coli*.

The specificity of *E. coli* adhesion is different from that of the oral bacteria [Ofek and Doyle, 1994; Offek, 1996]. For example, many of the characterized oral adhesins are resistant to mannose while being sensitive to galactose or other carbohydrates. Hence, it is impossible to infer from the inhibition studies performed with *E. coli*, the behavior of the cranberry preparation in tests which include oral bacteria. It was therefore unexpected to find that PF-1 had activity against oral bacteria.

In initial work, PF-1 was tested for its ability to inhibit (by addition of the PF-1 preparation to one of the bacterial pairs before mixing with the other) or to reverse (by its addition to performed coaggregates) the coaggregation of selected bacterial pairs including members of the following bacterial species: *Gemella morbillorum*; *Streptococcus oralis*; *Streptococcus sanguis*; *Actinomyces israelii*, *Actinomyces naeslundii*; *Capnocytophaga ochracea*, *Capnocytophaga sputigena*; *Prevotella intermedius*, *Porphyromonas gingivalis*; *Fusobacterium nucleatum* and *Actinobacillus actinomycetemcomitans*. For most of the pairs, the lowest concentration of PF-1 needed for inhibition was in the range of 50 to 10 $\mu\text{g/ml}$, whereas for reversal of coaggregation it was in the range of 125 to 200 $\mu\text{g/ml}$. Coaggregation of several pairs (e.g. *F. nucleatum* and *A. naeslundii*) was reversed by the cranberry PF-1 although they were resistant to all carbohydrates tested. The juice-derived material did not reverse the coaggregation of a number of pairs tested including members of *Streptococcus*, *Fusobacteria*, *Capnocytophaga* and *Actinomyces*, which indicates the specificity of the inhibitor.

As shown in Table 4, more extensive testing with non dialyzable material (NDM) from cranberry (which contains PF-1) showed the same pattern. Of a total of 37

bacterial pairs tested, 29 (78.1%) pairs showed reversal of intergeneric oral bacterial adhesion (coaggregation) by the NDM. The NDM was used at a concentration of ≤ 2500 $\mu\text{g/ml}$. Thirteen (36%) did not have the coaggregation reversed.

The above discussion provides a factual basis for the preparation and method of use of an isolated adhesion inhibitory fraction from cranberry juice. The methods used with and the utility of the present invention can be shown by the following non-limiting examples and accompanying figures.

EXAMPLES

GENERAL METHODS:

Reagents: Cranberry juice as available commercially is used and cranberry concentrated material (CCM) obtained directly from Ocean Spray Cranberries Inc., Lackeville-Middleboro MA 02349 is used for the isolation of PF-1. Bacterial strains used are of human gingival crevice origin (P. Kolenbrander, NIDR, NIH). All bacteria were grown at 37°C under anaerobic conditions (GasPack Anaerobic System, BBL) in Schaedler broth with the exception of *F. nucleatum* PK1594 which is grown either in Schaedler broth or brain heart infusion broth (BBL). Cells are harvested, washed with coaggregation buffer (CAB: 0.001 M Tris, 0.0001 M CaCl_2 , 0.0001 M MgCl_2 ,

0.15 M NaCl, 0.02% NaN₃, adjusted to pH 8.0) and stored at 4°C until used.

Visual Coaggregation Assay [Kolenbrander, 1988; Kolenbrander et al, 1989; Kolenbrander et al, 1993; Weiss et al, 1987]: Cell suspensions are adjusted to optical density of 1.5 at 400 nm (UV-Vis Spectrophotometer, Shimadzu) corresponding to approximately 10⁸ cells/ml. The appropriate partners (see Table 4; Figure 4) of 50μl each are mixed together vigorously for 10 seconds. A visual rating scale of 0 to 4 is used to grade the reactions. 0 = evenly turbid suspension with no visible aggregates indicating no coaggregation; 1 = few coaggregates; 2 = coaggregates formed remain in suspension; 3 = aggregates form big clumps and precipitate out of solution but fluid remain opaque; and 4 = maximal clumping leaving a clear supernatant.

Visual coaggregation assay to screen coaggregation inhibitors:

Inhibition of coaggregation was assayed by pre-incubating 40 μl of either one of the two test bacterial species with 20 μl of serial dilutions of the cranberry fraction for 30 minutes followed by adding 40 μl of the other bacterial species. The coaggregation is scored as described above.

Reversal of coaggregation was assayed by incubating 40 μl of bacterial suspension of one species with 40 μl

of bacteria of another species. The mixture is incubated for 30 minutes with constant agitation followed by adding 20 μ l serial dilutions of cranberry fraction. After further incubation of 15 minutes, the coaggregation is scored.

Haemagglutination (HA): *E. coli* IHE expressing P-fimbriae, grown on TSA agar at 37°C for 48 hours are harvested into PBS buffer and the bacterial suspension is two-fold serially diluted in 96 well (U-shaped) microtitration plates (50 μ l/well). To each well 25 μ l of 5% human erythrocyte (group A) suspension is distributed. After a 30 minute incubation at room temperature, the highest dilution of the bacterial suspension causing HA is determined so that the minimal HA unit is determined for use in testing samples.

To serial two-fold dilutions of samples (50 μ l/well of microtitration plate), 50 μ l of the bacterial suspension (6 minimal HA units) is added and the mixture is incubated at 37°C. HA is assayed on glass slides by mixing 50 μ l of the sample-bacteria mixture from each well with 25 μ l of 5% suspension of human group A erythrocytes. HA was recorded after 5 minutes at room temperature. HA inhibition (the highest dilution needed to complete inhibition of HA) is recorded according to control containing PBS instead of the tested sample.

ELISA for determination of adhesion inhibitory
activity of cranberry fractions against P-fimbriated
bacteria is as described in Figure 3 and in Example 2
herein below.

5 NMR was undertaken using a Bruker AMX-360 and ARX-
500 spectrometers.

EXAMPLE 1

ISOLATION OF PF-1

10 As an exemplar, the isolation of an anti-microbial
adhesion inhibitory fraction (PF-1) from cranberry juice
(cocktail or concentrate) was undertaken generally as
diagramed in Figure 1.

15 Isolation From Cranberry Juice Concentrate

Concentrated cranberry material (CCM; 500 ml),
known as juice concentrate, is dialyzed against 5 liter
distilled water (changed twice daily) for eight days.
The commercial source was generally Ocean Spray, but
20 other commercially available sources can be used.
Dialysis tubing is from Spectrum Medical Industries,
Inc., 60916 Terminal Annex, Los Angeles 90054.
Applicants used Molecular cut off 12000-14000, diameter
28.6 cm (6.4 vol./cm; Catalog No. 132680).

25 The material remaining in the dialysis tube (non
dialyzable material-NDM) is collected and lyophilized to

powder, generally yielding 20g. The NDM is used in some Examples as described herein. The NDM is solubilized with phosphate buffered saline (PBS) pH 7.5, 0.00M Phosphate.

5 BioGel P-60 beads (BioRad; 16 gram pre-swelled with PBS for 2 days at room temperature) are used to build a column of 2.4x100 cm. The column is washed. The void volume of the column is 350 ml and the column is equilibrated with 750 ml PBS. Three grams of dry NDM is
10 dissolved in 150 ml PBS and loaded on the column.

Colored material in NDM is bound by the beads. The column is washed exhaustively with PBS, which removes colored material and polysaccharides. Distilled water (750 ml) is then added and all material eluted is
15 collected, dialyzed against distilled water and lyophilized. The material eluted is denoted as purified fraction 1 (PF-1). Generally about 300 mg dry weight PF-1 is obtained. It is stored at room temperature. In an alternative embodiment the material is eluted with
20 ammonia (100 ml 0.1M) and the purified fraction denoted as PF-2 and dialyzed as for PF-1. As shown in Table 8, PF-2 performed in the assays similarly to PF-1, but there was a higher yield than PF-1, and PF-2 had a 2.5 to 5 fold increase as compared to NDM.

25

Isolation from Cranberry Juice Cocktail

5 The starting material was a commercially available
cranberry juice or juice concentrate. The commercial
source was generally Ocean Spray, but other commercially
available juice sources can be used. The method is
similar to the isolation from concentrate.

10 About 1.5 liters of the juice (or 25 ml of the
concentrate) is dialyzed against double distilled water
for 6 days at room temperature in dialysis tubing with a
molecular weight cutoff of 12,000 to 14,000. The
dialysate is lyophilized and approximately, one gram of a
nondialyzable material (NDM) is obtained.

The NDM is dissolved in 150 ml of PBS at pH 7.0,
clarified by centrifugation and loaded onto a column (4 x
10 cm). A polyacrylamide resin is used, BioGel P-60.

15 The "wash-through" fraction with PBS (containing
polysaccharides) is discarded and the active fraction is
eluted from the column with water. This eluted aqueous,
salt-free fraction is lyophilized yielding a reddish
powder, PF-1. In general the yield of PF-1 is
20 approximately 100 mg. When eluted with ammonia the
fraction is denoted as PF-2.

The isolates of PF-1 and/or PF-2 are standardized by
their anti-adhesion/aggregation activity, utilizing
hemagglutination assay, ELISA and the visual
25 coaggregation assay.

EXAMPLE 2ANALYSIS OF ACTIVITY

Upon isolation the PF-1 fraction (and/or NDM, PF-2)
5 is analyzed/quantitated for their anti-
adhesion/aggregation activity, utilizing the
hemagglutination assay, ELISA and the visual
coaggregation assay allowing both a functional
determination and an antigenic determination. These
10 assays determine the minimal concentration of the
fraction (dilution) needed of the material (fraction) to
provide inhibition of P-fimbriated bacterial adhesion to
human erythrocytes (HA) or coaggregation of the oral
bacteria. Table 1 provides exemplar data. (see also
15 Table 8)

Activity is also measured utilizing a quantitation
of inhibition by use of a modified ELISA protocol (Figure
3) as described herein below.

20 Materials

Microtitration plates (flat bottom, Costar); Human
Erythrocytes "Koscielak Ghosts" prepared according to
procedure described by D.J. Anstee & M.J.A. Tanner
(1974). 3.5 ml. of material is obtained from 10 ml of
25 packed erythrocytes, divided into aliquots and stored
at -70°C until use.

Bacteria: *E. coli* IHE expressing P-fimbriae, grown on TSA agar at 37°C for 48 hours are harvested into PBS buffer and the concentration determined according to Optical Density (O.D.). The bacterial suspension is stored in aliquots at -20°C until use.

Rabbit antiserum against *E. coli* IHE is prepared as described herein above. Anti-rabbit IgG antibody conjugated to Horseradish peroxidase, from donkey (Amersham). Substrate OPD tablets (Sigma). PBS-Phosphate Buffered Saline, Bovine Serum albumin (BSA), Methyl alcohol (analytic) H₂O₂.

Procedure

A. Determination of bacterial binding

1. Ghosts are distributed (100μl) in microtiter plate wells and dried at 37°C overnight. Concentrations are according to titration curve.
2. Blocking with BSA in PBS (200 μl/well) for 1 hour at 37°C. Washing 2x with PBS.
3. Binding of bacteria: Various concentrations of the bacterial suspension in BSA-PBS are distributed into the wells (100 μl/well) and incubated at 37°C for 1 hour. Washing 5x with PBS (gently).
4. Fixation: Methanol (100 μl/well) added and incubated at room temperature for 10 minutes. Washing 1x with PBS.

5. Antiserum diluted according to titration curve, in BSA-PBS, according to titration, 100 μ l/well, incubation at 37°C for 30 minutes. Washing 5x with PBS.
6. Conjugated anti-rabbit IgG antibody diluted according to manufacturer's instruction, 100 μ l/well, incubation at 37°C for 30 minutes. Washing 5x with PBS.
7. Substrate: 1 tablet in 25 ml. buffer (according to manufacturer's instruction), containing 10 μ l of 30% H₂O₂, 100 μ l/well, incubation at 37°C followed by 10-15 minutes at room temperature.

B. Titration of inhibitor

Bacterial suspension in concentration according to titration curve, incubated (vol/vol) at 37°C for 1 hour with various concentrations of a potential inhibitor (diluted in water) and the mixtures are distributed into the wells (100 μ l/well), as described in step 3 of the procedure herein above.

Bacteria incubated with PBS instead of inhibitor serve as control.

Recording of the results

O.D. values, read after subtraction of O.D. value of the antiserum control (all elements of the assay, except bacteria) are proportional logarithmically to the number of bacteria bound.

Percent of inhibition is calculated from the difference between the number of bacteria bound in the presence and in the absence of inhibitor.

The minimal concentration of the inhibitor causing 50% inhibition is calculated according to the linear regression analysis of the results. For procedure used for "translation" of the O.D. values into the number of the bacteria bound/well, see Athamna and Ofek (1988).

10 Testing of the system

Titration of bacteria dried in microtiter plates, in various concentrations (in water) with various concentrations of antiserum (AS) was tested. For further experiments AS diluted 1:150 was used. The minimal number of bacteria that can be detected was 5×10^4 /well.

Determination of optimal concentration of the ghosts

Ghosts in amounts: 100, 50, 20, 10, 5, and $2.5 \mu\text{l}$ in a final volume with water to $100 \mu\text{l}$ /well were dried overnight (see step 1 of the procedure). Bacterial suspension in initial concentration 1×10^{10} /ml (O.D. 1.5 of the suspension diluted 1:10) was serially diluted and incubated, as described in step 3 of the procedure.

The ELISA values were dependent on the amount of ghosts immobilized in the microtiter plates. The range of $10\text{-}50 \mu\text{l}$ was tested. The binding signal was low on

ghosts 20 μ l and lower on 10 μ l/well; 50 μ l was optimal.

Titration of binding of the bacteria to 50 μ l ghosts.

Bacterial titration is shown in Figure 7 where known
5 amounts of bacteria were immobilized on the bottom of
microtiter plates. ELISA is performed as described above
and O.D. units can be plotted as a function of the log
number of bacteria in each well. A linear regression
curve including only values that increase proportionally
10 to the number of bacteria can be used. Such a standard
curve is then used to calculate the number of bacteria
adherent to the erythrocyte ghosts, with and without
treatment with PF-1 or other fractions, from the ELISA
values obtained in the experiment.

Inhibition of binding of bacteria with PF-1 2. According
to Figure 7, 5×10^4 to 5×10^8 bacteria can be detected.
Optimal concentration of bacteria to be used in the test
is 1×10^8 /well. Applicants found that PF-1 inhibited
20 binding of the P fimbriated bacteria in a dose related
manner where 90-100% inhibition of binding was obtained
with 2mg/ml of PF-1 (Figure 8). For greater sensitivity,
the assay conditions are modified so that 50% inhibition
can be achieved by lower concentrations of PF-1.

EXAMPLE 3

ANALYSIS OF PF-1

PF-1 is acid precipitable in 2N HCl and can be redissolved in water without loss of activity. However, acid treatment (0.01 N HCl, 100°C, 30 minutes) results in reduced activity and not complete loss. It is not hydrolysed even in 2N HCl after 4 hours at 100°C.

Elemental analysis (Chemical Services, Organic Chemistry Department, Hebrew University of Jerusalem) of PF-1 after drying *in vacuo* at 60°C gave the following range:

carbon 43 - 51%

hydrogen 4 - 5%

No nitrogen, sulfur or chlorine

PF-1 gives a positive reaction in the phenol-sulfuric acid test [Dubois et al, 1956] suggesting the presence of carbohydrate of about 15% by weight using D-glucose as the standard.

EXAMPLE 4

FURTHER ANALYSIS OF PF-1

PF-1 was obtained as described in Example 1 and outlined in Figure 1. It exhibited adhesion-inhibitory activity on P-fimbriated bacteria and oral bacteria down to a concentration of 10-25 µg/ml.

Previous attempts to analyze PF-1 by high resolution nuclear magnetic resonance (NMR) were unsuccessful due to

severe line-broadening in the spectra (Figure 2A). In an attempt to obtain interpretable spectrum, the following experiments were done.

One reason for the observed broadening could be the presence of bound iron. Attempts were, therefore, made to remove bound iron from PF-1. For this purpose PF-1 (100 mg/ml) was dissolved in 10% solution of methanol, 8-hydroxy quinoline (an iron chelating agent) was added (5mg/ml) and the mixture was stirred for 3 hours at room temperature. The precipitate formed was removed by filtration or centrifugation and the methanol-aqueous solution was evaporated. The residue was dissolved in DMSO-d₆ (10 mg/ 0.5 ml) and examined by NMR.

A sharp line spectrum was obtained (Figure 2B). It shows that, as suspected, bound iron was masking the resolution of compounds in NMR. The iron free material was subject to acid metanolysis to obtain breakdown products of low molecular weight. The NMR spectrum of the products revealed that PF-1 contains inter alia phenyl rings (e.g. phenol rings), phenyl-CO groups, double bonds CH₂-chains and hydroxymethines and methylenes (Fig. 1C).

Further analysis of PF-1 (100 mg) was undertaken by extraction with butanol (10 ml) and then with ethylacetate (10 ml). No residues were found upon evaporation of the organic phases. Examination of dried

material obtained from the aqueous phase by NMR gave a spectrum identical qualitatively and quantitatively with that of the starting material. These data demonstrate that PF-1 is devoid of any fraction soluble in the above
5 organic solvents.

PF-1 was dissolved in 10% solution of methanol and loaded on Sephadex LH-20, P-10 or Dianion HP-20 columns. All of the material in PF-1 was adsorbed to the beads of the columns and could not be eluted with water.

10 PF-1 (50 mg) was acetylated by addition of pyridine (2ml) and acetic anhydride (2ml) followed by warm sonification for 2 hours. After incubation overnight at room temperature the solvents were evaporated to yield poly-acetylated PF-1. The NMR spectrum of the product
15 shows that the acetylation removed bound iron from some compounds, and the presence of acetyl groups (peaks around 2ppm). While the nonacetylated starting material was retained in silica gel column, the acetylated product passed through the column, however, no single compound
20 was obtained.

Acid methanolysis of PF-1 was performed by refluxing PF-1 (50 mg) in methanol (10 ml) and trifluoroacetic acid (2 ml) for 5 to 10 hours. The solvents were then removed by evaporation, the acid-methanolysed product was
25 dissolved in 10% methanol solution and the iron removed as described above. The NMR spectrum of the iron free

methanolysis product reveals sharp lines which denote saccharide like units, suggesting that acid methanolysis caused changes in the molecule. (See also Table 7 for anti-adhesion/aggregation activity of these fractions.)

5

EXAMPLE 5

COMPARATIVE ANALYSES

As discussed herein above, United States patent 5,474,774 to Walker et al issued December 12, 1995 does
10 disclose an extract from cranberry which is enriched for an activity which inhibits bacterial adhesion to surfaces. Applicants have compared PF-1 to the material of the '774 patent with the following results.

As described in Example 3, PF-1 was dissolved in 10%
15 solution of methanol in water and loaded on Sephadex LH-20, P-10 or Dianion HP-20 columns. All of the material in PF-1 adsorbed to the beads of either column and could not be eluted with water and water-methanol mixtures. The results suggest that the active material in PF-1 is
20 different from that found in cranberry extracts described by '774 patent as shown in Figures 1 and 10 of the '774 patent and Table 1 of the '774 patent.

The adhesion inhibition compounds from cranberry fruit as described in '774 patent are low molecular
25 weight, readily dissolved in organic solvents (Figures 1 and 10, '774 patent) as well as WO 96/30033. To examine

whether such anti-adhesive compounds are present in
cranberry juice, applicants employed a cranberry
concentrated material (CCM; obtained from Ocean Spray)
which contains both the high and low molecular weight
5 compounds found in the cranberry juice.

Lyophilized CCM (100 mg) was extracted with butanol
and ethylacetate as described for PF-1 (see Example 1
above). Most of the adhesion-inhibitory activity is
retained in the aqueous phase. The NMR spectra of CCM
10 fractions dissolved in the organic solvents and those
retained in the aqueous phases were determined. Well
resolved sharp peaks of various low molecular weight
compounds are observed in the butanol and ethyl acetate
fractions. These fractions lacked adhesion inhibitory
15 activity (Table 2). Inhibition was tested as described
previously (Zafriri et al, 1989) using P fimbriated *E.*
coli and human erythrocytes as target cells for adhesion
assays. For each fraction, listed in Tables 1 and 2 the
concentration in $\mu\text{g/ml}$ needed to inhibit hemagglutination
20 of guinea pig erythrocytes caused by P-fimbriated *E. coli*
and inhibition of coaggregation of oral bacteria was
determined. NDM and PF-1 were obtained as described in
Example 1.

The material retained in the aqueous phase also
25 contained well resolved low molecular weight compounds as
well as a substance(s) of very broad line spectrum

reminiscent of that observed in PF-1. The results support applicants' previous conclusion that the adhesion inhibitory activity in CCM is not soluble in organic solvents.

5 Table 3 summarizes the differences between the present invention and the material of the '774 patent.

 Further, PCT/US96/03978 (WO 96/30033) published application to Walker also discloses this extract from cranberry which is enriched for anti-adherence of
10 bacteria activity. However, analysis of the composition of PCT/US96/03978 provides further confirmation that it is not the composition of the present invention, PF-1. The composition of the '978 application appear to be tannins, the approximate molecular weight of the
15 disclosed compositions is 5000 although their mass spectrum gives a value of 577. The NMR spectra (proton and carbon) are different from those shown herein for the present invention.

 As discussed herein above, United States Patent
20 5,683,678 to Heckert et al discloses an oral composition containing anthocyanins isolated from cranberries and a composition having antiglucosyltransferase activity. Applicants have analyzed the composition of the present invention and it is not an anthocyanin and does not have
25 antiglucosyltransferase activity.

These references describe compositions which are rich in polyphenols usually found in tannins and anthocyanins and molecules with a molecular weight range of 200-10,000.

5 Polyphenol Assay: A modified Folin-Denis test [Folin and Denis, 1912] was used to detect all hydroxylated phenolic compounds [Mehansho et al, 1987]. As a control standard commercially available tannin and anthocyanin was obtained from Sigma Chemical Co. (St. Louis, MO).
10 The test was sensitive for phenolic compounds concentrations down to 0.02 mg/ml tanin in standard curves.

Results: NDM at 0.1mg/ml concentration (repeated twice) showed less than 0.02 mg/ml of phenolic residues.
15 Therefore, NDM does not contain phenolyic residues found typically in tannins and anthocyanins by this test.

Antiglucosyltransferase Assay: This assay is based on formation of a sucrose polymer (dextran) insoluble in methanol from sucrose. If NDM has
20 antiglucosyltransferase activity it will interfere with the assay.

Reagents: Glucosyltransferase enzyme solution was prepared from *S. sobrinus*, which produces four distinct glucosyltransferases, as follows. *S. sobrinus* 6715
25 bacteria were grown in defined medium [Terlecky] et al, 1975] for 16-18 hours. Cells were removed by

centrifugation and supernatants saved. The supernatants, on ice, were brought to 65% saturation with ammonium sulfate and stirred gently overnight. Precipitates were collected and dissolved in either 50mM NaCl (pH 5.7) or 20mM PO₄, 150 mM NaCl (pH 7.2). These precipitate samples provide a crude enzyme preparation. The samples are dialyzed against PBS and protein content assayed and adjusted to 1mg/ml to be used as the enzyme solution. Radiolabeled Sucrose-[¹⁴C] was purchased from ICN (Irvine, CA). The sucrose was kept frozen in water until use. For the assay an aliquot was withdrawn and diluted with unlabeled sucrose to give a final concentration of 50mM sucrose.

Assay: The assay described by Germaine et al [1974] was used with modification as follows. The enzyme solution is distributed to tubes (0.8 ml) and warmed to 37°C for 15-20 minutes. To one set of tubes NDM was added to a final concentration of 1 mg/ml. In a parallel set of control tubes no NDM was added. Sucrose-¹⁴C (0.2ml) was added to all tubes and the tubes incubated at 37°C. At timed intervals aliquots (0.1ml; 1 minute, 2 minutes, 5, 10, 15, 20, 25, 30 minutes) were removed from the NDM tubes and control tubes and placed onto Whatman 3MM filter disks. The filter disks were placed in cold methanol for 15 minutes to remove sucrose, the methanol

wash repeated and the disks air dried and counted in a scintillation counter.

Results: In two separate assays, no difference was observed between the control and NDM tubes. The
5 glucosyltransferase activity which catalyses synthesis of polysaccharide from sucrose was not altered by the presence of NDM.

EXAMPLE 7

10 EFFECT OF PF-1 ON ADHESION OF ORAL BACTERIA

Most members of the bacterial genera in the dental plaque are capable of interacting with each other by specific mechanisms and these bacteria-bacteria
15 interactions are now considered to be of paramount importance in the development of dental plaque [Kolenbrander et al, 1993]. Applicants' present invention provides that interference in the coaggregation between one bacterial species with another will hamper
20 the development of dental plaque and that therefore in an embodiment PF-1 can be used as a medicament in prevention and/or treatment of plaque as shown in the Example herein below.

The oral cavity of mammals and of humans in
25 particular is rarely colonized by *E. coli* nor is this bacterial species known to be involved in any diseases of

the oral cavity. Therefore in order to study the effect of PF-1 on bacteria of the oral cavity, bacteria known to colonize the oral cavity were used as described herein below.

5 Initially the relationship between PF-1 anti-adhesion activity on P-fimbriae of uropathogenic *E. coli* and the anti-aggregation activity of selected coaggregating pairs of bacteria was established. Coaggregating pairs were selected to represent the
10 bacteria that are involved in the various stages of dental plaque development and maturation. They included representatives of early colonizers (*S. oralis* 34 and *A. naeslundii* T14V); transition between early and late colonizers (*A. naeslundii* PK984 and *F. nucleatum* PK1909);
15 and potentially periopathogenic bacteria (*P. gingivalis* and *F. nucleatum* PK1594).

 In the first set of experiments, NDM (which contains PF-1) was tested to determine its effect on adhesion of oral bacteria. In these assays 40 pairs of bacteria that
20 have been shown to inhabit the oral cavity were tested. The pairs were allowed to adhere to each other by forming aggregates followed by adding NDM to determine the minimal concentration required to completely reverse the coaggregation (adhesion; see Figure 4). The results of
25 the experiments performed are summarized in Table 4 herein below.

As shown in Table 4, the coaggregation of some oral bacteria pairs was highly sensitive to NDM. The coaggregation was reversed by concentrations as low as 1250 $\mu\text{g/ml}$.

5 The coaggregation of other bacterial pairs were less sensitive or insensitive (coaggregation was not reversed by concentrations as high as 5000 $\mu\text{g/ml}$). These results indicate that the various bacteria bind the inhibitor with various degrees of affinity. There is a wide range
10 of receptor specificity of the adhesins carried by oral bacteria which is reflected in this finding.

In the second set of experiments PF-1 was employed and the results summarized in Table 5. As shown in Table 5, PF-1 was about ten times more active on a weight basis
15 in reversing coaggregation between *A. naeslundii* and *F. nucleatum* as compared to NDM, showing that most of the inhibiting activity in NDM resides in PF-1. Adsorption of PF-1 with P fimbriated *E. coli* diminished the ability of the absorbed fraction to reverse coaggregation of the
20 oral bacteria pair.

Table 5 also shows that alkaline treatments of PF-1 or acid treatment reduced the coaggregation activity of PF-1 while heating to 100°C has no effect. Previous studies have shown that acid treatment of PF-1 has no
25 effect on its anti-*E. coli* adhesion activity. The acid

modified PF-1 loses its ability to bind to oral bacteria but retains its ability to bind P fimbriated *E. coli*.

Table 6 shows that significantly lower amounts of NDM were needed to inhibit coaggregation by preincubating the fraction with either bacterial species of *F.*

nucleatum PK1909 and *A. naeslundii* PK984 pair of bacteria as compared to those needed to reverse preformed

coaggregation between the bacterial pair. The results also show that the affinity of the inhibitor in NDM

(which contains PF-1) to *F. nucleatum* is the same as that to *A. naeslundii* because the same NDM concentrations were needed to inhibit coaggregation when NDM was preincubated with either bacterial species. In contrast, NDM was more active in inhibiting coaggregation between *A. naeslundii* and *C. sputigena* when it was preincubated with the latter as compared to its inhibitory activity when preincubated with the former.

In Table 7, the anti-adhesion/aggregation activity of fractions from Example 3 are provided. The minimal inhibitory activity in $\mu\text{g/ml}$ against P-fimbriated bacteria is compared to oral bacteria for each fraction. In general there was agreement. However as discussed above the fraction eluted in methanol did not have anti-inhibitory activity.

In summary, the adhesion (coaggregation) of 29 out of 37 pairs tested is completely reversed, showing that

PF-1 will disturb the dental plaque. A hypothesis for the above observations can be made, but it is not to be construed as limiting the present invention to this one mode of action. The results are in line with the notion that the polymeric PF-1 targets its inhibitory activity to a defined range of bacterial adhesions. This notion is also evident from the analysis of the results of specific pairs of oral bacteria. For example, the adhesion of all 8 partners to *Fusobacterium nucleatum* PK 1904 is reversed by NDM, whereas that of only half of the partners to a different strain (PK 1594) of the same species is reversed by NDM. These results are analogous to *E. coli*, which produce multiple adhesins and only some of which, mainly those produced by the uropathogens, are inhibited by NDM.

EXAMPLE 8

Further Method of Purification

For additional purification of PF-1 utilizing gel filtration the method as described herein above was modified as follows: cranberry juice concentrate (UPC# 94040 Lot #95071) obtained from Ocean Spray, (200 ml) was dialyzed against distilled water for seven days (changing the water twice daily), using dialysis tubing with M.W. cut off of 12-15 kDa. The retentate was lyophilized to

yield 3.44 g (1.7% w/v) of non-dialysable material (NDM) as described herein.

The NDM was then fractionated on Bio-Gel P-60 beads (BioRad, 130±40mm, exclusion limit of 60 kDa). The beads
5 were soaked in PBS overnight and packed in a column 20 cm x 2.5 cm and the column was loaded with 20 ml (10mg/ml in PBS) NDM. All the material bound to about 5 cm of the top of the column. The column was washed with 100 ml PBS (at a flow rate of 1 ml/min.) and the water eluate
10 discarded. Upon passing through the column 100 ml of 0.1M NH₄OH, a second fraction was eluted. It was dialyzed against distilled water (1 liter) for 24 hours, lyophilized and the product designated PF-2. The minimal *E.coli* hemagglutination inhibitory concentration of PF-2
15 was 60-125 µg/ml, similar to that of PF-1 and represents an increased yield of about 2.5 to 5 fold as compared to NDM. The yield of PF-2 was 20-40% (w/w) which is considerably higher than that of PF-1 which was about 10% as described herein.

20

EXAMPLE 9

Inhibition of heamagglutination between *H. pylori* and human erythrocytes by NDM.

H. pylori strain 25 was used in the experiments
25 reported herein. It is a clinical isolate obtained from the Ezra Health Laboratory (Haifa, Israel). The strain

is maintained by growing on Columbia agar (Oxoid Ltd., Basingstoke, Hants, UK) supplemented with yeast extract 0.2% and 10% egg yolk emulsion in plates under microaerophilic conditions in anaerobic jars (CampyPakPlus, Beckton Dickinson Microbiology System, Cockeysville, MD) at 37°C. After three days bacteria are harvested from the agar plates for use. The bacteria are suspended in PBS to 1.0 O.D. at 660 nm which is equivalent to approximately 5×10^8 CFU/ml.

As described for the hemagglutination assay, bacteria were grown on agar for three days, scraped from agar and suspended in phosphate buffer saline to a density of 1.0 OD. Hemagglutination activity and its inhibition by NDM were done as described previously (Zafriri et al, 1989). The results are shown in Table 9 and demonstrate that NDM will inhibit heamagglutination by *H. pylori*.

EXAMPLE 10

Inhibition of Adhesion of Helicobacter pylori to human gastric mucus by NDM

Preparation of microtiter plates containing immobilized human mucus: Human mucin (S-11/97) was obtained from the Department of Pathology, RamBam Hospital, Haifa. The mucin was dissolved in 0.1 M NaCl

to obtain a 10% (V/V) solution. 1 M NaOH was added to reach pH 7.0 and incubated overnight at 4°C. The suspension was then centrifuged for 10 minutes at 10,000 xG and to the sediment mucin ethanol 60% (V/V) was added.

5 After 30 minutes at 4°C, the mucus was sedimented by centrifugation and re-dissolved in 0.1 M NaCl to a concentration of 1µg/ml protein. Coating of wells with mucin was done by adding 0.1 ml of mucin to each well of 96-microtiter plates, incubating for 24 hours at 37°C and
10 washing with 20 mM phosphate buffer saline (PBS) [Burger et al, 1998].

Determination of adhesion of *H. pylori* to immobilized mucin and its inhibition by NDM: 0.01 ml NDM (1 mg/ml) in PBS or PBS only was added to 0.1 ml
15 bacterial suspension and the mixture was placed into the wells containing the immobilized human mucin. After 90 minutes incubation the wells were washed free of non-adherent bacteria and the amount of bacteria remaining adherent was determined by measuring urease activity of
20 the bound bacteria. Urease activity was monitored over three hours time by adding to each well at the desired time phenol red (7 mg/ml) and urea (330 mico mol /ml) followed by recording the development of color at 560 nm. Control experiments showed that NDM has no effect on the
25 urease activity of the bacteria and that preincubation of

the mucin layer with NDM followed by washing off excess NDM had no effect on *H. pylori* adhesion.

The results (Figure 9) show the urease activity of *H. pylori* bound to mucin after incubation with and without NDM, as described above. It can be seen that very little activity was detected in wells incubated with the bacteria in the presence of 0.1 mg/ml NDM. Therefore there was reduced adhesion of the *H. pylori* to the human mucin.

Throughout this application, various publications, including United States patents, are referenced by citation or number. Full citations for the publications are listed below. The disclosures of these publications and patents in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The invention has been described in an illustrative manner, and it is to be understood that the terminology which has been used is intended to be in the nature of words of description rather than of limitation.

Obviously, many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood that within the scope of the appended claims, the invention

may be practiced otherwise than as specifically described.

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Table 1

Inhibition of Coaggregation/Adhesion Studies

Fraction	Minimum Adhesion-Inhibitory Conc.* HA: P-fimbriated	Minimum Adhesion-Inhibitory Conc.* Coag: Oral Bacteria
Cranberry Juice	1:8 - 1:16	n.d.
Non-dialyzable material (NDM)	50-200 $\mu\text{g/ml}$	50 -200 $\mu\text{g/ml}$
PF-1	10-40 $\mu\text{g/ml}$	12-50 $\mu\text{g/ml}$

* Dilution or concentration ($\mu\text{g/ml}$) needed to complete inhibition of hemagglutination (HA) or coaggregation (Coag) of oral bacteria.

TABLE 2

Inhibition of Coaggregation/Adhesion Studies

Fraction	HA* ($\mu\text{g/ml}$)	Coag* ($\mu\text{g/ml}$)
PF-1	10-40	12-50
Unfractionated CCM	400-1600	500-1000
Butanol Soluble	3000	1000-2000
Ethyl acetate soluble	3000	500-1000
Retained in aqueous phase	400	500-1000

* See legend to Table 1.

TABLE 3

PROPERTY OF MATERIAL	PF-1	'774 Patent and PCT/03978
Molecular Weight/Size	High/Big	Low/Small
Method to Obtain	Dialysis and gel filtration	Extraction by organic solvents
Solubility in organic solvents	Not soluble	Soluble
Target System	Dental Plaque	Oral Surfaces
Target Bacteria	Oral Bacteria	Not specified
Bacteria Tested	Oral Bacteria (>20 species) and P-fimbriated	E. coli
Effect on adhesion of Type 1 P-fimbriated bacteria	None	Positive
Ability of bacteria tested to reside in oral cavity	All	None
Type of effect on adhesion	Reversal and Inhibition	Inhibition
Adhesion system tested	Interspecies and intraspecies adhesion of oral bacteria	E. coli adhesion to erythrocytes and bladder cells
Relevance of systems tested to dental hygiene	Relevant	Not relevant

TABLE 4
REVERSAL OF COAGGREGATION

Table 4: Effect of nondialysable material (NDM)⁺ obtained from cranberry on intergeneric adhesion (coaggregation) of oral bacteria

Bacterial partners tested		Concentration of NDM (mg/ml) needed to <u>reverse</u> coaggregation*
<i>Fusobacterium nucleatum</i> PK1909	<i>Actinomyces naeslundii</i> PK984	1.25
	<i>Actinomyces naeslundii</i> PK29	1.25
	<i>Gemella morbilorum</i> PK509	1.25
	<i>Actinomyces naeslundii</i> PK947	2.50
	<i>Streptococcus sanguis</i> J22	>2.50
	<i>Porphiromonas gingivalis</i> PK1924	2.50
	<i>Streptococcus oralis</i> SS34	2.50
<i>Fusobacterium nucleatum</i> PK1904	<i>Actinomyces israelii</i> PK14	2.50
	<i>Actinomyces naeslundii</i> PK947	1.25
	<i>Capnocytophaga ochracea</i> ATCC33596	1.25
	<i>Actinomyces naeslundii</i> PK29	2.50
	<i>Gemella morbilorum</i> PK509	1.25
	<i>Prevotella denticola</i> PK1277	2.50
	<i>Actinomyces naeslundii</i> T14V	1.25
	<i>Actinomyces naeslundii</i> ATCC12104	1.25
<i>Fusobacterium nucleatum</i> PK1594	<i>Actinomyces naeslundii</i> PK29	2.50
	<i>Actinomyces naeslundii</i> PK984	2.50
	<i>Actinomyces israelii</i> PK14	2.50
	<i>Actinomyces naeslundii</i> PK947	2.50
	<i>Porphiromonas gingivalis</i> PK1924	2.50
	<i>Streptococcus gordonii</i> ATCC51656	1.25
	<i>Capnocytophaga ochracea</i> ATCC33596	1.25
	<i>Actinomyces naeslundii</i> T14V	1.25
	<i>Streptococcus oralis</i> SS34	>2.50
	<i>Actinomyces naeslundii</i> ATCC12104	>2.50
	<i>Actinomyces israelii</i> PK16	2.50

TABLE 4 (continued)

Table 4 (continued): Effect of nondialysable material (NDM) obtained from cranberry
on intergeneric adhesion (coaggregation) of oral bacteria

Bacterial partners tested		Concentration of NDM (mg/ml) needed to <u>reverse</u> coaggregation*
<i>Capnocytophaga sputigena</i> ATCC33612	<i>Actinomyces naeslundii</i> PK984	1.25
	<i>Actinomyces israelii</i> PK14	2.50
	<i>Actinomyces naeslundii</i> PK947	2.50
	<i>Actinomyces naeslundii</i> PK29	2.50
	<i>Actinomyces naeslundii</i> ATCC12104	>2.50
<i>Actinobacillus</i> <i>actinomycetemcomitans</i> JP2	<i>Actinomyces naeslundii</i> PK984	>2.50
<i>Prevotella intermedius</i> PK1511	<i>Actinomyces naeslundii</i> PK984	1.25
<i>Actinomyces naeslundii</i> PK947	<i>Capnocytophaga sputigena</i> ATCC33612	>2.50
	<i>Streptococcus oralis</i> SS34	>2.50
<i>Prevotella loescheii</i> PK1295	<i>Actinomyces naeslundii</i> PK984	>2.50
	<i>Actinomyces israelii</i> PK14	>2.50

*> not completely inhibited at 2.50mg/ml

TABLE 5

Table 5: Effect of various treatments on the ability of cranberry-derived fractions to reverse coaggregation between *Fusobacterium nucleatum* PK1909 and *Actinomyces naeslundii* PK984

<u>Treatment of Cranberry fraction</u>	<u>Concentration (mg/ml) of cranberry-derived fractions needed to reverse coaggregation</u>
NDM, untreated	1.25
PF-1, untreated	0.15
PF-1, 100°C, 30 minutes	0.15
PF-1, 0.1 N HCl, 30 minutes	1.25
PF-1, 0.1 N NaOH, 30 minutes	1.25
Trypsin, 100µg/ml, 30 minutes	0.15
PF-1, adsorbed with P. fimbriated <i>E. coli</i>	>1.25*
*lack of inhibitory activity at 1.25 mg/ml	

TABLE 6

Table 6 Comparison between the ability of cranberry-derived PF-1 to inhibit and to reverse coaggregation of oral bacteria

Coaggregating Bacterial pairs		PF-1 fraction		PF-1 (mg/ml) needed to inhibit or reverse coaggregation
A	B	Preincubated with: A	Added to performed coaggregates B	
<i>A. naeslundii</i> PK984	<i>F. nucleatum</i> PK1909	+	+	0.037 0.037 1-5.0
<i>A. israelii</i> PK14	<i>C. sputigena</i> ATCC33612	+	+	0.15 0.075 2-5.0

TABLE 7

Minimal Inhibitory Activity (MIA; $\mu\text{g/ml}$) Obtained from Iron free PF-1 and Other Fractions Obtained by Reverse Phase Chromatography, Acetylation and Acid Methanolysis.

Fraction	Treatment	MIA of P-fimbriated bacteria	MIA of Oral bacteria
PF-1	Untreated (Fig. 2A)	10 - 25	12 - 50
PF-1-20 Iron free	8-hydroxyquinoline (Fig. 2A)	25 - 35	25 - 50
	Reverse Phase Chromatography of PF-1-20:		
PF-1-20/1	water elution	40	50
PF-1-20/2-5	20% - 80% methanol	300-2000	50-2000
PF-1-31	Acetylation	NT	>400
PF-1-26	Acid Methanolysis (8 hours) (Fig. 2C)	200-600	100-220
PF-1-37	Acid Methanolysis (30 hours)	200-600	100-300

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TABLE 8

ACTIVITY OF CRANBERRY-DERIVED FRACTIONS

Fraction	Minimal inhibitory concentration mg/ml	
	<i>E.coli</i> P adhesion	Oral bacteria coaggregation.
NDM	0.200-0.150	0.200-0.150
PF-1	0.125-0.050	0.120-0.050
PF-2	0.125-0.050	0.120-0.050

TABLE 9

Effect of PF-1 on adhesion of *Helicobacter pylori*

Strain	Source	Hemaggl- utinating titer	Concentration of PF-1 needed to inhibit hemagglutination mg/ml
#25	Clinical isolate	1:16	0.25

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